

MO-4890

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1 in an atmosphere of CO<sub>2</sub>:O<sub>2</sub>N<sub>2</sub> (8:8:84). Supernat was removed and  
the cells were treated with 0.2% KCL for 5 min. and 0.1% KCL for 25 min.  
The KCL was removed and the cells were harvested by scraping. The  
harvested cells were passed through a 22 gauge needle to break down  
5 the cell structure. The cell lysate was subjected to low speed  
centrifugation for 10 min. and the semi-purified organisms remaining in the  
supernatant were harvested by high speed centrifugation. Antigen was  
pooled from 25 flasks and a portion of the antigen was subjected to a  
french press treatment for the production of soluble antigen. The  
10 Reminder was aliquoted and stored at -70°C. This soluble antigen was  
formulated into a vaccine according to the following procedure. Vaccine  
antigen was formulated with TITERMAX® adjuvant or Freund's Incomplete  
adjuvant at a concentration of 500ug of antigen/dose. With the TITERMAX®  
adjuvant, 0.5mL was mixed with 0.5mL of antigen to produce a 1.0mL  
15 dose containing 500ug of antigen. With the Freund's Incomplete adjuvant,  
2.0 mL of adjuvant was mixed with 2.0 mL of antigen such that the total  
dose also contained 500ug.

In order to determine whether the antigen produced could protect  
pigs from a homologous challenge or from exposure to heterologous  
20 isolates or strains, ten 4-week-old pigs were vaccinated and later  
challenged. Ten control pigs received equal doses of a mock vaccine  
which contained only the tissue culture medium Minimal Essential Medium  
(MEM)) and adjuvant (without antigen). The vaccine used for the first  
vaccination contained TITERMAX® adjuvant while the vaccine used for the  
25 second vaccination contained Freund's Incomplete adjuvant. Serum  
samples were taken prior to vaccination (prebleed), at day of booster  
(Day 14) and at the day of challenge (Day 35) to demonstrate the  
production of an immune response post vaccination. Serum was tested  
for antibody to *L. intracellularis* via an ELISA wherein the wells in a 96-well  
30 plate were coated with *L. intracellularis* antigen (purified from pig gut  
epithelial cells) of a clinical isolate which was from a different source than  
the isolate used to produce the vaccine. Therefore, presence of an